comprising the step of administering to newborns and infants an infant formula comprising at least one casein or fragments thereof selected from the group consisting of naturally occurring, recombinant, synthetic animal or vegetable caseins not containing the sequences: Pro-Gly-Pro-Ile-His (SEQ ID NO:1) and Pro-Gly-Pro-Ile-Pro (SEQ ID NO:2).

36. A method for the prevention of insulin-dependent diabetes comprising the administration to newborns and infants a milk which does not contain caseins containing the sequences: Pro-Gly-Pro-Ile-His (SEQ ID NO:1) and Pro-Gly-Pro-Ile-Pro (SEQ ID NO:2), said casein being obtained by the following steps: providing a vector suitable for the expression of the casein; transfecting said vector in a cell selected from the group consisting of prokaryotic cell, unicellular eukaryotic cell or a cell derived from a multi cellular organism; and isolating and purifying said casein.

Kindly cancel claims 21-27.

REMARKS

The objection to claim 23 and claims 23-25 has been rendered moot by the cancellation of those claims.

Claims 21 - 27 were rejected under 35 U.S.C §101 on the basis that the claims were drawn to non-statutory subject matter. Claims 21 - 27 have been canceled and new claims 28-36 have been drafted to avoid the rejection based on non-statutory subject matter. The new claims no longer encompass naturally occurring human or non-human milk. New claim 28 points out a dietary or pharmaceutical product that contains recombinant or a synthetic casein which is distinct from naturally occurring casein. The product, as claimed, does not contain the amino acid sequences Pro-Gly-Pro-Ile-His (SEQ ID NO:1) and Pro-Gly-Pro-Ile-Pro (SEQ ID NO:2). The language of new claim 28 and claims dependent on claim 28 clearly define a product that is not found in nature and, therefore, the claims are not subject to rejection based as non-statutory subject

matter. New claim 29 explains the manner in which such sequences could be avoided. New claim 29, corresponding to former claim 23 has been amended in order to point out the invention.

New claim 30, corresponding to former claim 22 has been amended to point out that the caseins do not contain the specific sequences identified by the applicant.

The new method claims (claims 33-36) pont out the claimed invention by reciting the specific steps that are employed. Claims 32 and 36 are supported in the text because PCT application WO 93/04171 is incorporated by reference into the present application. In particular, the steps involving gene expression and protein isolation are referenced at page 5, line 14 as being described in the PCT application WO 93/04171. This document is to be incorporated by reference in the present application in order to support the additions contained in newly submitted claims 32 and 36 and referring to the production of recombinant casein. Claims 32 and 36 are based on claims 13-16 of WO 93/04171, which recite the steps of obtaining a recombinant protein.

New claims 33-35 have been worded in order to recite a method for the prevention of insulin-dependent diabetes. For these reasons, it is requested that this ground of rejection be withdrawn.

Claims 21-27 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Reconsideration is requested.

The Examiner, cited Cavallo *et al.* and Atkinson *et al.* to support the rejection under § 112 (first paragraph). The present ground of rejection is based on a statute that requires an applicant for a patent to provide sufficient information that would enable one skilled in the art to make and use the invention. No issues has been raised with regard to the enabling nature of the present application which

contains detailed information as to how to make the product of the invention. The information as to how to use the product is implicit in the disclosure that the modified casein is used as an infant food in the same manner that unmodified casein is used. Thus there cannot be any serious question regarding the fact that the applicant has taught the art how to make and use the invention.

The thrust of the Examiner's arguments is that "the specification does not provide any teachings that show that administration of the product as claimed would prevent or inhibit the onset of IDDM".

The relationship of 35 U.S.C.§101 and 35 U.S.C.§112, first paragraph is spelled out in MPEP§2164.07. All that is required by 35 U.S.C.§101 is that some use for the claimed invention must be set forth in the specification. This has been done in the present specification. The requirements of 35 U.S.C.§112, first paragraph may only be properly used to reject patent claims if one skilled in the art could not practice the claimed invention based on the disclosure. The Examiner has urged that an unreasonable amount of experimentation would be required for one to "make and/or use the claimed invention and methods of using the same" (Office Action, page 6). The Examiner has also urged that the "specification does not provide any teachings which show that the administration of the product as claimed would prevent or inhibit the onset of IDDM".

The Examiner's contentions with regard to an unreasonable amount of experimentation have nothing to do with the directions for use of the claimed invention. The invention is a food for newborns and infants and since the prior art methods of feeding of casein based foods to newborns and infants is completely analogous to the feeding of the modified caseins of the present invention, no undue experimentation is required to use the claimed invention.

The present rejection appears to be a rejection that is based on 35 U.S.C.§101 where the Examiner is calling for proof that the claimed invention is effective in preventing

diabetes. The provisions of 35 U.S.C.§101 have been interpreted by the Federal Circuit as being applicable only when a patent application is "totally incapable of achieving a useful result". Brooktree Corp. v. Advanced Micro Devices, Inc., 24 USPQ2d 1401,1412 (Fed. Cir.1992). All that is required under 35 U.S.C.§101 is that some beneficial function flow from the disclosure. MPEP§2107. In the present case, the products are intended for nutrition and no issue has been raised regarding the fact that the modified casein will provide some nutrition to a host, i.e. an infant. This establishes that there can be no valid "how to use" objection to the claimed subject matter.

The present application teaches: sequence homology (at least 90%) between 63-68 of bovine beta-casein and human GLUT 2; this sequence of bovine beta-casein elicits an immune response via production of anti beta-casein antibodies and lymphocytes which, by cross reactivity, are directed towards the homologous sequence of GLUT 2, causing damage to the cells that produce insulin; and non-human milk derived products free of these beta-casein sequences are not immunogenic with respect to the GLUT 2 protein because of the absence of such sequence homology and therefore will not induce an autoimmune attack on the insulin-producing cells of the pancreas.

The specification of the present application teaches the removal of diabetogenic non-human beta-caseins from the food that is to be ingested by a susceptible population. The Examiner's rejection indicates that the specification's teaching of the removal of a diabetogenic substance cannot enable a method for preventing diabetes. In the medical arts, the question of prevention does not require a showing that non-exposure to a potentially dangerous substance does not produce damage. In medicine, when the hypothesis is made that a substance can induce or provoke an affliction, based on documented epidemiological studies which indicate that such substance could be harmful, the first remedy to be taken is to find an effective means to prevent the exposure to said substance. The present application teaches an effective means

for preventing exposure of a diabetogenic substance which is useful even if 100% prevention of IDDM is achieved.

Atkinson et al. points out three main areas of investigation into the pathogenesis of IDDM, one of which, early infant diet, encompasses the present invention. The referenced section only calls into question the theory that there is any one single cause of IDDM. Nothing in Atkinson et al. teaches that removal of immunogenic hexapeptide sequences from non-human beta-casein is counterproductive in preventing IDDM.

Moreover, although the Examiner cites Atkinson, et al. as teaching that "it is not clear if beta-casein is a modifier of the disease pathogenesis or a trigger of the onset of IDDM," Atkinson et al. actually supports a "more complex" role of dietary factors such as non-human beta-casein in the pathogenesis of IDDM, and does not include anything that can be cited as a basis on which to challenge the teachings of the present application.

The Applicant wishes the Examiner to consider the following recent references, as showing the state of the art with regard to the present view of the cow's milk hypothesis in causation of IDDM:

"...the lower fraction of A1 and B beta caseins in Icelandic cow's milk may explain why there is lower incidence of Type 1 diabetes in Iceland than in Scandinavia," Pediatrics 106:719-24,2000;

"... breast feeding prevents the generation of antibody response to bovine beta casein. This findings may have relevance for disease prevention," Diabetes Metab Res Rev 17:1-4, 2001;

See also: Diabetologia 1999 Mar; 42(3):292; Diabetes 49:912-7, 2000; Diabetes 49:1657-65, 2000; Diabetologia 44:63-9, 2001; Proc Nutr Soc 59:573-9, 2000; J Am Coll Nutr 19:176-190, 2000.

Applicant does not take issue with the Atkinson, et al. hypothesis, that several environmental factors may be implicated in the pathogenesis of Type 1 diabetes. Nonetheless, exposure to cow's milk, and in particular, exposure to beta casein fractions is the most common agent to which susceptible individuals are exposed.

The Examiner argues further that: "in light of the many different factors that could potentially be involved in the pathogenesis of IDDM, it could not be predictably concluded that beta-casein causes the observed immune response in humans." This observation confuses two separate issues. The first issue is that there may be many potential factors involved in the pathogenesis of IDDM. Again, Applicant does not dispute this contention, and merely states that the present application points only to one factor that has been discovered to play a role in the pathogenesis of IDDM. The second issue is whether it could be predictably concluded that beta-casein causes the observed immune response in humans.

The Examiner has questioned the teachings in Cavallo et al. This reference discloses that more than half of the diabetic patients (not the totality) showed an immune response to beta-casein derived from cow's milk. It should be pointed out that in medical research, it is not the typical case where there is a finding that a particular disease has a single cause. Even so, whereas Type 1 diabetes is a multifactorial disease, the 51.1% finding becomes even more persuasive evidence implicating the role of cow's milk and beta-casein's as potent antigens which are capable of inducing an autoimmune response involving cross reaction with insulin producing cells in the pancreas, which leads to the destruction of insulin producing cells.

On page 7 of the Office Action, the Examiner states that the Applicant has argued that there is a strong association between cow's milk and the incidence of IDDM in children, but provides no evidence to support the association. The Applicant hereby provides the attached copies of documents demonstrating that exposure to cow's milk in early infancy

increases risks of Type 1 diabetes, particularly in children who are genetically susceptible to diabetes. The applicant maintains that this data provides straightforward evidence of the value of avoiding exposure to such casein.

On page six of the Office Action, the Examiner argued that in the claim drawn to a product with non-human casein substituted with homologous sequence of human beta-casein, it would be expected tat a sequence homologous to the hexapeptide sequence would also elicit an immune response.

The present application teaches that the amino acid sequences of non-human beta-casein have been identified and isolated, and that the corresponding human beta-casein sequence has also been identified and isolated. The application, teaches that non-human beta-caseins are responsible for the immune response in humans. The application teaches that the immunogenic sequence in non-human beta-casein is homologous to GLUT 2 protein regions and that is the basis for the molecular mimicry involved in the autoimmune destruction of the pancreatic cells. The present application does not teach that the human and non-human sequences are homologous or that the human beta-caseins elicit an immune response in humans. Attached to this Amendment are several published scientific papers which indicate that cow's milk consumption is positively correlated with Type 1 diabetes incidence. These articles demonstrate that there is a correlation between onset of diabetes and beta-casein consumption in early infancy. The more pertinent phrases have been highlighted in yellow in the submitted documents and corresponding relevant citations have been hightlighted in yellow in the complete articles.

Attached hereto is a declaration signed by the inventor, Paolo Pozzilli, which provides evidence that lymphocytes from Type 1 diabetics show reactivity to beta-casein which cross-reacts with the beta cell antigen GLUT 2. The data provides further evidence that: 1) beta-casein is a major antigen in Type 1 diabetes; 2) its elimination from cow's milk represents a fundamental step towards prevention of the autoimmune

response leading to Type 1 diabetes; and 3) environmental factors, such as cow's milk proteins, are capable of activating "ignorant" auto reactive T cell clones by means of antigens that mimic the host proteins.

The Declaration also provides evidence of the acceptance of the role of beta casein in the causation of Type 1 diabetes by the existence of two major trials which are being carried out to confirm that Type 1 diabetes may be prevented by providing a cow's milk hydrolysate at birth for individuals susceptible to Type 1 diabetes.

The first one, called Prevefin, is being carried out in Italy. In this clinical trial infants with high genetic risk of developing diabetes as possessing the HLA genotype DR3/DR4 receive in their diet a cow's milk hydrolysate where beta casein is not present. A control group where ordinary cow's milk is administered acts for comparison. The outcome of the study is represented by the generation 1-3 years later of specific antibodies to pancreatic beta cells, a well recognized immunological sign which indicates progression versus full blown disease. The trial is supported by major agencies in Italy, including the Ministry of Health and the Ministry of University & Technological Science.

The other trial, called TRIGR, is a large worldwide study involving 42 centers. This is the largest trial in terms of financial support that has been ever implemented in the field of diabetes. It is sponsored by the National Institutes of Health in the United States, the Juvenile Diabetes Research Foundation (USA) and the European Union. The approach is similar to Prevefin in that infants (nearly 3,000) of mothers or fathers with a family history of Type 1 diabetes are randomized to a cow's milk hydrolysate deprived of beta casein or ordinary milk as control.

These trials are being carried out as a direct consequence of the present application and reinforce the scientific importance and usefulness of the findings therein disclosed. Depending on the results, primary prevention will possibly become a major breakthrough in human health.

In addition, for the Examiner's convenience, the following flow-chart (Annex 13) is attached in which the Applicant provides a self-explanatory summary of the art surrounding his invention. An Appendix is attacheds which provides summar ies of each of the attached literature references which are being submitted as evidence that beta-casein is a factoir in the causation of Type 1 diabetes. For these reasons, it is requested that the rejection under 35 U.S.C.§112, first paragraph be withdrawn.

The novelty of the present invention resides in having found that bovine beta-casein, and particularly the sequences Pro-Gly-Pro-Ile-His (SEQ ID NO: 1) and Pro-Gly-Pro-Ile-Pro (SEQ ID NO: 2) elicit diabetes owing to molecular mimicry with the protein GLUT2. Therefore, a feeding formula and a method are provided to prevent diabetes in infants and newborn.

One aspect of the invention resides in feeding infants and newborn with a feeding product, such as a milk, which contains bovine beta-caseins artificially deprived of the sequences Pro-Gly-Pro-Ile-His (SEQ ID NO: 1) and Pro-Gly-Pro-Ile-Pro (SEQ ID NO: 2). Such product may additionally contain various other caseins which are not immunogenic in that they lack SEQ ID NO: 1 and 2. Other conventional components may be present in the product, such as lipids, vitamins, minerals and so on.

Another aspect of the invention resides in a method of preventing diabetes characterized in that a feeding product such as a milk is administered to infants and newborn, said milk or product being characterized in that it contains caseins in which the sequences Pro-Gly-Pro-Ile-His (SEQ ID NO: 1) and Pro-Gly-Pro-Ile-Pro (SEQ ID NO: 2) are absent or have been modified or removed.

None of the above elements are contained in or suggested by the prior art cited against the present invention by the Examiner.

Claims 21, 22, 23, and 25 were rejected under 35 U.S.C. 102(b) as being anticipated by Freidman (U.S. Patent No. 4,501,585. These claims have been cancelled and this rejection

is rendered moot. Moreover, the newly submitted claims not anticipated by Friedman. The submitted product claims are based on the presence of bovine beta-caseins. Friedman, on the other hand, teaches to feed newborn with the human milk collected with the harvesting device therein disclosed. Moreover, Friedman is silent about a method for preventing diabetes.

Claims 21 and 23 were rejected as being anticipated by Shimatani. Claims 21 and 23 were cancelled, thereby rendering this ground for rejection moot. Moreover, the newly submitted claims are not anticipated by Shimatani. Shimatani teaches the preparation of a desalted milk starting from naturally occurring milk, specifically cow milk, among others (col. 2, lines 43-44). Such milk, which is used as whole milk, contains caseins with the sequences considered immunogenic by the applicant. Therefore, Shimatani uses beta-caseins which are different from the applicant's and therefore Shimatani does not anticipate or make obvious the present invention. Moreover, Shimatani is silent about a method for preventing diabetes.

Claim 24 was rejected as being anticipated by Rosen (U.S. Patent No. 5,304,489). Claim 24 has been cancelled, therefore, this ground for rejection is rendered moot. Moreover, the newly submitted claims are not anticipated by Rosen. Rosen teaches the production of transgenic mice expressing rat beta-casein but is silent about the modified bovine beta-caseins of the present invention. Therefore new product claims should not be considered anticipated. Moreover, Rosen is silent about a method for preventing diabetes.

Claims 26-27 are rejected as being anticipated by Bergstrom et al. (WO 93/04171, published 3/4/93). Claims 26 and 27 have been cancelled, therefore this basis for rejection is rendered moot. Moreover, the newly submitted claims are not anticipated by Bergstrom. The newly presented product claims always contain modified beta-casein and this aspect renders novel the product claims in view of Bergstrom which

discloses to obtain recombinant human beta-casein. Moreover, Bergstrom teaches a milk having "calcium binding activity, opioid activity, angiotensin converting enzyme (ACE) inhibitory activity" (see Bergstrom page 4, lines 16-19); such activities have nothing to do with diabetes. For this reason the method claims are not anticipated by Bergstrom.

Claims 26 and 27 were rejected as being anticipated by Slattery (U.S. Patent No. 5,795,611). Claims 26 and 27 have been cancelled, therefore this basis for rejection is rendered moot. Moreover, the new claims are not anticipated by Slattery. The product claims always contain modified bovine beta-casein and this aspect renders novel the product claims in view of Slattery, which discloses a composition based on human alpha-lactalbumin and human beta-casein. Moreover Slattery teaches the preparation of a composition to be used for solving "digestibility and allergenic problems" (see Slattery col. 2, lines 19-22); such problems have nothing to do with diabetes and therefore the newly presented method claims are to be considered novel in view of Slattery.
For these reasons, it is requested that the prior art of record not be applied to reject the newly presented claims.

An early and favorable action is earnestly solicited.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on 5/08/02

APPENDIX

In the following, citations are quoted in italics and the references therein incorporated are in capital letters.

Annex 1 (abstract) Diabetologia 1999 Mar;42 (3):292- *Elliot RB "Total protein consumption did not correlate with diabetes incidence (r=+0.402), but consumption of the beta-casein A1 variant did (r=+0.726). Even more pronounced was the relation between beta-casein (A1+B) consumption and diabetes (r=+0.982)"*

Annex 2 (abstract) J Am Coll Nutr 2000 Apr;19 (2Suppl):176S - 190S *Schrezenmeir J et al.*

"…T-cell and humoral responses related to cow's milk proteins were suggested to trigger diabetes."

Annex 3 (abstract) Diabetes 2000 Oct:49 (10):1657-65 *Paronen J et al.*

"…first immunization to insulin occurs by exposure to bovine insulin (BI) in cow's milk (CM) formula" "At the age of 3 months, both cellular and humoral responses to BI were higher in infants exposed to CM formula than in infants fully breast-fed (P=0.015 and P=0.007)."

Annex 4 (abstract) Pediatrics 2000 Oct;106 (4):719-24 *Thorsdottir I et al.*

"The analyses of milk samples showed that the percentage of the A1 and B variants of beta-casein in Icelandic milk was significantly lower than in the milk from the Scandinavian countries. …The lower fraction of A1 and B beta-caseins in Icelandic cow's milk may explain why there is a lower incidence of IDDM in Iceland than in Scandinavia."

Annex 5 (abstract) Proc Nutr Soc 2000 Nov;59 (4):573- *Wasmuth HE et al.*

"Cow's milk-based infant formulas and cow's milk consumption in childhood have been suggested to promote the development of type 1 diabetes mellitus … introduction of cow's milk-based infant formula within the first 3 months of life is associated with increased risk of type 1 diabetes mellitus. … cow's milk proteins

may provide mimicry epitopes relevant in autoimmunity, as well as destabilizing oral tolerance mechanisms by biologically active peptides."

Annex 6 (abstract) Diabetologia 2001 Jan;44 (1):63-9 *Kimpimaki T et al.*

"This study aimed to establish the relation between early infant nutrition and signs of beta-cell autoimmunity in young children. … These observations suggest that short-term breastfeeding and the early introduction of cow's milk-based infant formula predispose young children who are genetically susceptible to Type 1 diabetes to progressive signs of beta-cell autoimmunity."

Annex 7 (abstract) Diabetes Metab Res Rev 2001 Jan-Feb;17 (1):51-4 *Monetini L et al.*

"Bovine beta-casein is a cow's milk protein that targets both humoral and cellular immune responses in patients with Type 1 diabetes and. to a lesser degree, also in normal subjects. … Elevated levels of beta-casein antibodies were found in bottle-fed infants compared to breast-fed infants ($p<0.0001$). … By western blot analysis we confirmed specific binding to bovine beta-casein in bottle-fed infants, in children with Type 1 diabetes and in controls exposed to cow's milk, but not in infants who were exclusively breast-fed. … Breast-feeding within the first 4 months of life prevents the generation of antibody response to bovine beta-casein despite the mother's consumption of cow's milk during the breastfeeding period. These findings may have relevance for disease prevention."

Annex 8 (abstract) Pediatr Clin North Am 2001 Feb;48 (1):125-41 *Davis MK*

"Increased risks for type 1 diabetes … have been associated with artificial infant feeding and short-term breastfeeding."

Annex 9 (complete article) Diabetologia (1999) 42: 292-296 *Elliot RB et al*

"Previous studies have shown a strong correlation between the consumption of milk and the incidence of diabetes (SCOTT FW COW

MILK AND INSULIN-DEPENDENT DIABETES; IS THERE A RELATIONSHIP? *AM J CLIN NUTR* 1990;51:489-491 - DAHL-JORGENSEN K ET AL . RELATIONSHIP BETWEEN COW' S MILK CONSUMPTION AND INCIDENCE OF IDDM IN CHILDHOOD *DIABETES CARE* 1991;14:1081-1083 - ELLIOT RB EPIDEMIOLOGY OF DIABETES IN POLYNESIA AND NEW ZELAND *PEDIATR ADOLESCENT ENDOCRINOL* 1992;21:66) *Countries that had a low milk consumption such as Japan had a low diabetes incidence, whereas those with a high consumption such as Finland, had a high diabetes incidence." "The countries used in this study were selected only if they could provide appropriate and reliable data for the calculation of beta-casein variant consumption and for the incidence of diabetes." "Iceland was an unusual point because it had both the highest milk protein consumption and the lowest A1 and B values out of the selected countries. The strong correlation between total milk protein consumption and Type 1 diabetes (when excluding Iceland) agrees with the findings from other studies* (SCOTT FW COW MILK AND INSULIN-DEPENDENT DIABETES; IS THERE A RELATIONSHIP? *AM JCLIN NUTR* 1990;51:489-491 - DAHL-JORGENSEN K ET AL . RELATIONSHIP BETWEEN COW' S MILK CONSUMPTION AND INCIDENCE OF IDDM IN CHILDHOOD *DIABETES CARE* 1991;14:1081-1083 - ELLIOT RB EPIDEMIOLOGY OF DIABETES IN POLYNESIA AND NEW ZEALAND *PEDIATR ADOLESCENT ENDOCRINOL* 1992;21:66) . *The relatively low proportion of beta-casein A1 in Icelandic milk could account for the low incidence of childhood diabetes despite their very high consumption of milk. Anedoctically, the Masai people of Africa have had until recent times a very large intake of cow milk from early infancy* (ARTHEM K PASTORAL MAN IN THE GARDEN OF EDEN. UPPSALA RESEARCH REPRINTS IN CULTURAL ANTHROPOLOGY, ACCESSION NO. 572.967 8A69 UNIVERSITY OF UPPSALA, DEPARTMENT OF CULTURAL ANTHROPOLOGY P 73) , *yet a very low incidence of diabetes in childhood (Dr. M Jacobson, Doctor in Charge, Selian Lutheran Hospital, Arusha, Tanzania, personal communication). Their herds consisted of Bos Indicus cows which produce a low protein milk containing predominantly beta-casein A2* (NG-KWAI-HANG KF ET AL. GENETIC POLYMORPHISM OF MILK PROTEINS. IN: FOX PF (ED) ADVANCED DAIRY CHEMISTRY, VOL. 1:PROTEINS ELSEVIER APPLIED SCIENCE, LONDON, PP 405-455) ."

Annex 10 (complete article) Diabetes Vol. 49., oct. 2000 1657-1665 *Paronen J et al*

"*We have previously shown that exposure to cow's milk (CM) formula elicits antibody formation to insulin in some children* (VAARALA O ET AL. COW'S MILK FORMULA FEEDING INDUCES PRIMARY IMMUNIZATION TO INSULIN IN INFANTS AT GENETIC RISK FOR TYPE 1 DIABETES 1999;48:1389-1394 - VAARALA O ET AL. COW MILK FEEDING INDUCES ANTIBODIES TO INSULIN IN CHILDREN: A LINK BETWEEN COW MILK AND INSULIN DEPENDENT DIABETES MELLITUS? *SCAND J IMMUNOL* 1998;47:131-135)" "*The main emphasis of the present study was to analyze the development of T-cell immunity to insulin in the second pilot of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR), in which infants with a first-degree relative with Type 1 diabetes and increased genetic risk for the disease were randomized to receive either an adapted CM-based formula or an extensively hydrolyzed casein (HC)-based formula after breast-feeding until the age of 6-8 months. The emergence of cellular immunity to insulin was measured by proliferation test in a group of 56 children, and the development of insulin-binding antibodies was measured by EIA and RIA in 119 children in relation to CM exposure and family history of Type 1 diabetes*" " *We observed that oral exposure to BI in the CM formula induced insulin-specific T-cell and antibody responses in infants at increased risk for Type 1 diabetes. This finding is in accordance with our previous observations on the introduction of insulin-binding antibodies by early CM feeding* (VAARALA O ET AL. COW'S MILK FORMULA FEEDING INDUCES PRIMARY IMMUNIZATION TO INSULIN IN INFANTS AT GENETIC RISK FOR TYPE 1 DIABETES 1999;48:1389-1394 - VAARALA O ET AL. COW MILK FEEDING INDUCES ANTIBODIES TO INSULIN IN CHILDREN: A LINK BETWEEN COW MILK AND INSULIN-DEPENDENT DIABETES MELLITUS? *SCAND J IMMUNOL* 1998;47:131-135)"

Annex 11 (complete article) Pediatrics 106(4): 719 *Thorsdottir et al.*

"***Objective*** *To compare children with insulin-dependent diabetes mellitus (IDDM) with controls in Iceland regarding their consumption of cow's milk in infancy, and to investigate the*

beta-casein fractions in Scandinavian and Icelandic cow's milk. The A1 variant of beta-casein has been shown to be diabetogenic in animal studies, and suggestions have been made that the B variant of beta-casein acts similarly. Differences in the relative proportions of beta-casein might explain the lower incidence of IDDM in Iceland than in Scandinavia" "studies from Europe and the United States have shown a relationship between short duration of breast feeding and a higher incidence of IDDM (BORCH-JOHNSEN K, JONER G, MANDRUP-POULSEN T, RELATION BETWEEN BREAST-FEEDING AND INCIDENCE RATES OF INSULIN-DEPENDENT DIABETES MELLITUS: A HYPOTHESIS. *LANCET* 1984; 2:1083-1086 - MAYER EJ, HAMMAN RF, GAY EC, LEZOTTE DC, SAVITZ DA, KLINGENSMITH GJ REDUCED RISK OF IDDM AMONG BREAST-FED CHILDREN. *DIABETES* 1988; 37:1625-1632)" *"... a meta-analysis has pinpointed exposure to cow's milk as an important determinant of IDDM,* (GERSTEIN HC COW'S MILK EXPOSURE AND TYPE 1 DIABETES MELLITUS: A CRITICAL OVERVIEW OF THE CLINICAL LITERATURE *DIABETES CARE* 1994; 17:13-19) *and in 1994 the American Academy of Pediatrics found reasos to strongly recommend breastfeeding. They also recommended that families with a strong history of IDDM should avoid products containing intact cow's milk protein during the first year of life.* (DRASH AL ET AL. INFANT FEEDING PRACTICES AND THEIR POSSIBLE RELATIONSHIP TO THE ETIOLOGY OF DIABETES MELLITUS: WORK GROUP ON COW'S MILK PROTEIN AND DIABETES MELLITUS *PEDIATRICS* 1994; 94: 752-754)" "*Animal studies show that A1 beta-casein is diabetogenic* (ELLIOT RB ET AL. THE ROLE OF BETA-CASEIN IN THE INTRODUCTION OF INSULIN-DEPENDENT DIABETES IN THE NON-OBESE DIABETIC MOUSE AND HUMANS. IN *SEMINAR ON MILK PROTEIN POLYMORPHISM: IDF SPECIAL ISSUE 9702.* BRUSSELS, BELGIUM: INTERNATIONAL DAIRY FEDERATION; 1997: 445-453) *, and suggestions have been made on a similar effect from B beta-casein* (ELLIOT RB ET AL. THE ROLE OF BETA-CASEIN IN THE INDUCTION OF INSULIN-DEPENDENT DIABETES IN THE NON-OBESE DIABETIC MOUSE AND HUMANS. IN *SEMINAR ON MILK PROTEIN POLYMORPHISM: IDF SPECIAL ISSUE 9702.* BRUSSELS, BELGIUM: INTERNATIONAL DAIRY FEDERATION; 1997: 445-453 - ELLIOT RB ET AL. TYPE 1 (INSULIN DEPENDENT) DIABETES MELLITUS AND COW MILK: CASEIN VARIANT CONSUMPTION *DIABETOLOGIA* 1999: 42:292-296)" *"A sequence homology between*

beta-casein and several beta-cell molecules has also been suggested as an immune response trigger (CAVALLO MG ET AL CELL-MEDIATED IMMUNE RESPONSE TO BETA-CASEIN IN RECENT-ONSET INSULIN-DEPENDENT DIABETES: IMPLICATIONS FOR DISEASE PATHOGENESIS *LANCET* 1996; 348:926-928)" *"An international, double-blind study is currently in progress in which infants at high risk for IDDM are given hydrolyzed infant formula for the first 6 months of life to avoid intact dietary protein* (SCHATZ DA ET AL PREVENTION OF INSULIN-DEPENDENT DIABETES MELLITUS;: AN OVERVIEW OF THREE TRIALS *CLEVE CLIN J Med* 1996;63:270-274)" *"Substantial evidence indicates that cow's milk is important for the development of IDDM"*

Annex 12 (complete article) Diabetes Metab. Res. Rev. 2001;**17**:51-54 *Monetini et al.*

"Results Elevated levels of beta-casein antibodies were found in bottle - fed infants compared to breast-fed infants ($p<0.0001$). Antibody levels to bovine beta-casein were also significantly higher in children with Type 1 diabetes compared to age-matched controls ($p=0.03$). By western blotanalysis we confirmed specific binding to bovine beta-casein in bottle-fed infants, in children with Type 1 diabetes and in controls exposed to cow's milk, but not in infants who were exclusively breast-fed." (Such results are shown in fig. 2 and fig. 3 of the article), High levels of antibodies to bovine β-casein were detected in bottle-fed infants under the age of 4 months, whereas exclusively breast-fed infants of the same age did not show an antibody response to this protein."*… our findings reinforce the concept that exposure to cow's milk should be avoided during the first months of life, at least in individuals at genetic risk for the disease, as recommended in a recent publication* (AMERICAN ACADEMIC OF PEDIATRICS. BREAST-FEEDING AND THE USE OF HUMAN MILK. *PEDIATRICS* 1997;**100**:1035-1039)

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